

**CLAIMS**

What is claimed:

- 5 1. A glycolipid-inserted-embryo for the preparation of an embryo modified to enhance the implantation of the embryo into the endometrium wherein:
- the glycolipid-inserted-embryo has an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
  - 10 • the glycolipid has been modified to incorporate a binding part wherein said binding part is adapted to enable binding to an attachment molecule.
2. An embryo as claimed in claim 1 wherein the glycolipid has been modified to incorporate the binding part prior to the insertion of the lipid tails into a cell
- 15 membrane of the embryo or into the zona pellucida of the embryo.
3. An embryo modified to enhance the implantation of the embryo into the endometrium wherein:
- the embryo has an attachment molecule which is capable of attaching to
  - 20 the endometrium; and
  - the attachment molecule is linked to the embryo by an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
  - the attachment molecule and the glycolipid have each been modified to
  - 25 incorporate a binding part so that the attachment molecule and the glycolipid are bound together via their respective binding parts either directly or through a bridging molecule.
4. An embryo as claimed in any one of claims 1 to 3 wherein the modification to
- 30 the glycolipid is to the carbohydrate portion of the glycolipid.

5. An embryo as claimed in any one of claims 1 to 4 wherein the attachment molecule is selected from the group consisting of carbohydrates or oligosaccharides, glycolipids, glycoconjugates, proteins, peptides, acyl groups or polymers.

5 6. An embryo as claimed in any one of claims 1 to 5 wherein the attachment molecule is selected from the group consisting of natural or synthetic carbohydrates or oligosaccharides, proteins or peptides including poly L-lysine, antibodies, lectins, polyvinyl pyrrolidine, and functionally equivalent derivatives thereof.

10 7. An embryo as claimed in any one of claims 1 to 6 wherein the attachment molecule is an immunoglobulin antibody.

8. An embryo as claimed in claim 7 wherein the attachment molecule is immunoglobulin G (IgG).

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9. An embryo as claimed in any one of claims 1 to 8 wherein the attachment molecule is adapted to interact with the epithelial cells of the endometrium, mucus, mucin, or other endogenous or exogenously provided component of the endometrium.

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10. An embryo as claimed in any one of claims 1 to 9 wherein the attachment molecule is an endometrial attachment molecule.

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11. An embryo as claimed in any one of claims 1 to 10 wherein the glycolipid is selected from the group consisting of phosphoglycerides and sphingolipids.

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12. An embryo as claimed in any one of claims 1 to 11 wherein the binding part of the glycolipid and the attachment molecule are bound together by simple non-covalent binding interactions including ionic, van de Waals, water exclusion, electrostatic, hydrogen bonding or chelation binding.

13. An embryo as claimed in any one of claims 1 to 11 wherein the binding part of the glycolipid and the attachment molecule are bound together by covalent bonding.
14. An embryo as claimed in any one of claims 1 to 10 wherein the attachment  
5 molecule and the glycolipid are bound together by avidin-biotin binding.
15. An embryo as claimed in claim 14 wherein the binding part of the glycolipid comprises biotin and the binding part of the attachment molecule comprises avidin.
- 10 16. An embryo as claimed in claim 14 wherein the binding part of the glycolipid comprises avidin and the binding part of the attachment molecule comprises biotin.
17. An embryo as claimed in any one of claims 1 to 11 wherein the attachment molecule and the glycolipid are bound together through a bridging molecule.
- 15 18. An embryo as claimed in claim 17 wherein the bridging molecule comprises avidin and the binding part of both the attachment molecule and the glycolipid comprises biotin.
- 20 19. An embryo as claimed in claim 17 wherein the bridging molecule comprises biotin and the binding part of both the attachment molecule and the glycolipid comprises avidin.
- 25 20. An embryo as claimed in any one of claims 1 to 11 wherein the attachment molecule and the glycolipid are bound together by a chelation interaction between at least one chelator and a chelated metal ion.
21. An embryo as claimed in claim 20 wherein the binding part of both the attachment molecule and the glycolipid comprises a chelator.

22. An embryo as claimed in claim 20 or claim 21 wherein the chelator is a poly-histidine recombinant or non-recombinant protein.
- 5 23. An embryo as claimed in any one of claims 20 to 22 wherein the chelator is attached covalently to the glycolipid.
24. An embryo as claimed in any one of claims 20 to 22 wherein the chelator is attached non-covalently to the glycolipid.
- 10 25. An embryo as claimed in claim 24 wherein the chelator is attached to the glycolipid by biotin or avidin.
26. An embryo as claimed in any one of claims 20 to 25 wherein the chelated metal ion is  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  or  $\text{Cu}^{2+}$ .
- 15 27. An embryo as claimed in any one of claims 1 to 26 wherein the lipid tails of the glycolipid are inserted into the cell membranes of the embryo.
28. An embryo as claimed in any one of claims 1 to 26 wherein the lipid tails of the glycolipid are inserted into the zona pellucida of the embryo.
- 20 29. An embryo as claimed in any one of claims 1 to 15, 17, 18 and 20 to 28 wherein the modified glycolipid is a biotinylated glycolipid.
- 25 30. An embryo as claimed in any one of claims 1 to 29 wherein the glycolipid is a glycolipid of the ganglioside class that contains sialic acid groups, or a glycolipid of the neutral class that contains galactose.
- 30 31. An embryo as claimed in any one of claims 1 to 30 wherein the attachment molecule is a molecule that has a binding affinity for molecules on cell membranes including the mucus coat of cell membranes.

32. An embryo as claimed in claim 31 wherein the molecules on cell membranes are receptor sites and/or blood group related antigens.
33. An embryo as claimed in claim 31 or claim 32 wherein the cell membranes are endometrial cell membranes.
34. A method of preparing a glycolipid-inserted-embryo including the step of:
- contacting a glycolipid with an embryo, where the glycolipid has been exogenously modified to incorporate a binding part, wherein said binding part is adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the modified glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo.
35. A method of preparing a modified embryo including the steps of:
- contacting an attachment molecule with a glycolipid, wherein the attachment molecule and the glycolipid have each been modified to incorporate a binding part adapted to enable the attachment molecule and the glycolipid to bind together via their respective binding parts either directly or through a bridging molecule; and then
  - contacting the attachment molecule bound to the glycolipid with an embryo so that the lipid tails of the glycolipid insert into the cell membranes of the embryo or into the zona pellucida of the embryo.
36. A method of preparing a modified embryo including the steps:
- contacting a glycolipid with an embryo wherein the glycolipid has been exogenously modified to incorporate a binding part, wherein said binding part is adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo to provide a glycolipid-inserted-embryo; and then

- contacting the glycolipid-inserted-embryo with an attachment molecule, wherein the attachment molecule has been modified to incorporate a binding part adapted to enable binding to the binding part of the glycolipid either directly or through a bridging molecule.

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37. A method as claimed in any one of claims 34 to 36 wherein the attachment molecule is selected from the group consisting of carbohydrates or oligosaccharides, glycolipids, glycoconjugates, proteins, peptides, acyl groups or polymers.

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38. A method as claimed in any one of claims 34 to 37 wherein the attachment molecule is selected from the group consisting of natural or synthetic carbohydrates or oligosaccharides, proteins or peptides including poly L-lysine, antibodies, lectins, polyvinyl pyrrolidine, and functionally equivalent derivatives thereof.

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39. A method as claimed in any one of claims 34 to 38 wherein the attachment molecule is an immunoglobulin antibody.

40. A method as claimed in claim 39 wherein the attachment molecule is immunoglobulin G (IgG).

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41. A method as claimed in any one of claims 34 to 41 wherein the attachment molecule is adapted to interact with the epithelial cells of the endometrium, mucus, mucin, or other endogenous or exogenously provided component of the endometrium.

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42. A method as claimed in any one of claims 34 to 41 wherein the attachment molecule is an endometrial attachment molecule.

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43. A method as claimed in any one of claims 34 to 42 wherein the glycolipid is selected from the group consisting of phosphoglycerides and sphingolipids.

44. A method as claimed in any one of claims 34 to 43 wherein the attachment molecule and the glycolipid are bound together by simple non-covalent binding interactions including ionic, van de Waals, water exclusion, electrostatic, hydrogen bonding or chelation binding.
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45. A method as claimed in any one of claims 34 to 43 wherein the attachment molecule and the glycolipid are bound together by covalent bonding.
46. A method as claimed in any one of claims 34 to 43 wherein the attachment molecule and the glycolipid are bound together by avidin-biotin binding.
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47. A method as claimed in claim 46 wherein the binding part of the glycolipid comprises biotin and the binding part of the attachment molecule comprises avidin.
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48. A method as claimed in claim 46 wherein the binding part of the glycolipid comprises avidin and the binding part of the attachment molecule comprises biotin.
49. A method as claimed in any one of claims 34 to 44 wherein the attachment molecule and the glycolipid are bound together through a bridging molecule.
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50. A method as claimed in claim 49 wherein the bridging molecule comprises avidin and the binding part of both the attachment molecule and the glycolipid comprises biotin.
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51. A method as claimed in claim 49 wherein the bridging molecule comprises biotin and the binding part of both the attachment molecule and the glycolipid comprises avidin.
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52. A method as claimed in any one of claims 34 to 44 wherein the attachment molecule and the glycolipid are bound together by a chelation interaction between at least one chelator and a chelated metal ion.

53. A method as claimed in claim 52 wherein the binding part of both the attachment molecule and the glycolipid is a chelator.

5 54. A method as claimed in claim 52 or claim 53 wherein the chelator is a poly-histidine recombinant or non-recombinant protein.

55. A method as claimed in any one of claims 52 to 54 wherein the chelator is attached covalently to the glycolipid.

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56. A method as claimed in any one of claims 52 to 54 wherein the chelator is attached non-covalently to the glycolipid.

57. A method as claimed in claim 56 wherein the chelator is attached to the glycolipid via biotin or avidin.

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58. A method as claimed in any one of claims 52 to 57 wherein the chelated metal ion is  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  or  $\text{Cu}^{2+}$ .

20 59. A method as claimed in any one of claims 34 to 58 wherein the lipid tails of the glycolipid are inserted into the cell membranes of the embryo.

60. A method as claimed in any one of claims 34 to 58 wherein the lipid tails of the glycolipid are inserted into the zona pellucida of the embryo.

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61. A method as claimed in any one of claims 34 to 47, 49, 50 and 52 to 60 wherein the modified glycolipid is a biotinylated glycolipid.

30 62. A method as claimed in any one of claims 34 to 61 wherein the glycolipid is a glycolipid of the ganglioside class that contains sialic acid groups, or a glycolipid of the neutral class that contains galactose.



- 5 63. A method as claimed in any one of claims 34 to 62 wherein the attachment molecule is a molecule that has a binding affinity for molecules on cell membranes including the mucus coat of cell membranes.
64. A method as claimed in claim 63 wherein the molecules on cell membranes are receptor sites and/or blood group related antigens.
- 10 65. A method as claimed in claim 63 or claim 64 wherein the cell membranes are endometrial.
66. A method of enhancing the implantation of an embryo into the endometrium of an animal including the steps:
- 15     • preparing a modified embryo according to the method of any one of claims 35 to 65; and
- transferring the modified embryo to the uterus of the animal.
67. A method as claimed in claim 66 including the step:
- 20     • introducing a component with which the attachment molecule will interact into the uterus of the animal so that the component becomes localised to the endometrium.
68. A method as claimed in claim 66 or claim 67 wherein the animal is a human or domesticated animal.
- 25 69. A method as claimed in claim 66 or claim 67 wherein the modified embryo is prepared from a species, hybrid or variety of animal different from the species, hybrid or variety of animal of the uterus.
- 30 70. A glycolipid-attachment molecule construct when used for generating a modified embryo comprising a glycolipid modified to incorporate a binding part and

an attachment molecule modified to incorporate a binding part wherein the respective binding parts are adapted to enable the modified glycolipid and the modified attachment molecule to bind to each other either directly or indirectly through a bridging molecule.

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71. A construct as claimed in any one of claims 70 wherein the modification to the glycolipid is to the carbohydrate portion of the glycolipid.

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72. A construct as claimed in claims 70 or 71 wherein the attachment molecule is selected from the group consisting of carbohydrates or oligosaccharides, glycolipids, glycoconjugates, proteins, peptides, acyl groups or polymers.

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73. A construct as claimed in any one of claims 70 to 72 wherein the attachment molecule is selected from the group consisting of natural or synthetic carbohydrates or oligosaccharides, proteins or peptides including poly L-lysine, antibodies, lectins, polyvinyl pyrrolidine, and functionally equivalent derivatives thereof.

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74. A construct as claimed in any one of claims 70 to 73 wherein the attachment molecule is an immunoglobulin.

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75. A construct as claimed in claim 74 wherein the attachment molecule is immunoglobulin G (IgG).

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76. A construct as claimed in any one of claims 70 to 75 wherein the attachment molecule is adapted to interact with the epithelial cells of the endometrium, mucus, mucin, or other endogenous or exogenously provided component of mucus.

77. A construct as claimed in any one of claims 70 to 76 wherein the attachment molecule is an endometrial attachment molecule.

78. A construct as claimed in any one of claims 70 to 77 wherein the glycolipid is selected from the group consisting of phosphoglycerides and sphingolipids.

5 79. A construct as claimed in any one of claims 70 to 78 wherein the attachment molecule and the glycolipid are bound together by simple non-covalent binding interactions including ionic, van de Waals, water exclusion, electrostatic, hydrogen bonding and chelation binding.

10 80. A construct as claimed in any one of claims 70 to 79 wherein the attachment molecule and the glycolipid are bound together by covalent bonding.

81. A construct as claimed in any one of claims 70 to 79 wherein the attachment molecule and the glycolipid are bound together by avidin-biotin binding.

15 82. A construct as claimed in claim 81 wherein the binding part of the glycolipid comprises biotin and the binding part of the attachment molecule comprises avidin.

20 83. A construct as claimed in claim 81 wherein the binding part of the glycolipid comprises avidin and the binding part of the attachment molecule comprises biotin.

84. A construct as claimed in any one of claims 70 to 80 wherein the attachment molecule and the glycolipid are bound together through a bridging molecule.

25 85. A construct as claimed in claim 84 wherein the bridging molecule comprises avidin and the binding part of both the attachment molecule and the glycolipid comprises biotin.

30 86. A construct as claimed in claim 84 wherein the bridging molecule comprises biotin and the binding part of both the attachment molecule and the glycolipid comprises avidin.

87. A construct as claimed in any one of claims 70 to 79 wherein the attachment molecule and the glycolipid are bound together by a chelation interaction between at least one chelator and a chelated metal ion.

5 88. A construct as claimed in claim 87 wherein the binding part of both the attachment molecule and the glycolipid comprises a chelator.

89. A construct as claimed in claim 87 or claim 88 wherein the chelator is a poly-histidine recombinant protein.

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90. A construct as claimed in any one of claims 87 to 89 wherein the chelator is attached covalently to the glycolipid.

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91. A construct as claimed in any one of claims 87 to 89 wherein the chelator is attached non-covalently to the glycolipid.

92. A construct as claimed in claim 91 wherein the chelator is attached to the glycolipid via biotin or avidin.

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93. A construct as claimed in any one of claims 87 to 92 wherein the chelated metal ion is  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  or  $\text{Cu}^{2+}$ .

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94. A construct as claimed in any one of claims 70 to 82, 84, 85 and 87 to 93 wherein the glycolipid modified to incorporate a binding part is a biotinylated glycolipid.

95. A construct as claimed in any one of claims 70 to 94 wherein the glycolipid is a glycolipid of the ganglioside class that contains sialic acid groups, or a glycolipid of the neutral class that contains galactose.

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96. A construct as claimed in any one of claims 70 to 95 wherein the attachment molecule is a molecule that has a binding affinity for molecules on cell membranes including the mucus coat of cell membranes.

5 97. A construct as claimed in claim 96 wherein the molecules on cell membranes are receptor sites and/or blood group related antigens.

98. A construct as claimed in claim 96 or 97 wherein the cell membranes are endometrial.

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99. A construct as claimed in any one of claims 70 to 97 wherein the attachment molecule is a molecule that has a binding affinity for molecules on embryo cell membranes or the zona pelludica.

15 100. A method of enhancing the implantation of an embryo into the endometrium of an animal including the steps of:

- introducing a construct as claimed in claim 99 into the uterus of the animal so that the construct becomes localised to the endometrium; and then
- transferring the embryo to the uterus of the animal.

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101. A kit for use in enhancing the implantation of an embryo of an animal comprising one or more preparations of a glycolipid-attachment molecule construct as claimed in any one of claims 70 to 99.